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THE EFFECT OF VARIOUS CHEMICAL SUBSTANCES ON THE HEMOLYTIC REACTION [*]

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Hektoen[1] in 1903 pointed out that some of the inorganic salts, such as $CaCl_2$, $MgCl_2$, $BaCl_2$, $NaSO_4$, $Na_2C_2O_4$, and $Na_2C_2H_4O_6$, inhibit the hemolytic reaction when present in certain concentrations. This work was extended by Manwaring[2] and by Hektoen and Ruediger[3] to cover bacteriolysins, as well as hemolysins, and a much larger number of salts. They found that small doses of M/8 solutions of many salts prevent the lysis of red corpuscles and of bacteria by various sera, and concluded that the antilytic action is the result of the action of these salts on the complements. Arkin[4] (1913), in reporting on the effect of several substances on opsonins, mentions that lactic acid in certain concentrations not only inhibits phagocytosis, but prevents complement from combining with amboceptor in the hemolytic reaction. I have noted in a previous report[5] that a temporary drop in complement occurs during the Eck-fistula operation, which seems to be the result of the anesthesia. It is generally known that an acidosis usually results from the administration of ether or chloroform,[6] especially following chloroform anesthesia, and perhaps this acidosis is a factor in causing the temporary drop in complement during Eck-fistula operations, inasmuch as the drop is much greater under chloroform than under ether anesthesia. Wendelstadt[7] showed that in the presence of a small amount of amboceptor glycogen suspends hemolysis by acting on the complement. Hektoen and Ruediger mention the work of Camus and Pagniez,[8] Neisser and Doehring,[9] and others, showing the ability of uremic serum to inhibit hemolysis by normal serum. Walker[10] and others have reported the presence of complement-fixing substances in the serum of a certain percentage of diabetics. Cumming[11] has shown that the sign of the Wassermann reaction may be changed by the influence of various salts, acids, and alkalies on the hemolytic system.

The work of this paper is an investigation of the effect of lactic acid, hydrochloric acid, sodium bicarbonate, and acetone on the complement content of rabbits in vivo; of ether and chloroform anesthesia

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[*] Received for publication November 16, 1916.

[1] Trans. Chicago Path. Soc., 1903, 5, p. 303.

[2] Jour. Infect. Dis., 1904, 1, p. 112.

[3] Ibid., p. 379.

[4] Ibid, 1913, 13, p. 408.

[5] Sherwood, Smith, and West, Jour. Infect. Dis., 1916, 19, p. 682.

[6] Woolley, Jour. Lab. and Clin. Med., 1916, 1, p. 782.

[7] Centralbl. f. Bakteriol., I. O., 1903, 34, p. 83.

[8] Camus and Pagniez, Compt. rend. Soc. de biol., 1901, 53, p. 730.

[9] Berl. klin. Wchnschr., 1901, 38, p. 593.

[10] Jour. Am. Med. Assn., 1916, 66, p. 488.

[11] Jour. Infect. Dis., 1916, 18, p. 151.

on the hydrogen-ion concentration and complement content of the blood of normal rabbits; and lastly of carbon dioxid, lactic acid, hydrochloric acid, uric acid, urea, benzoic acid, tartaric acid, hydrazin sulfate, acetic acid, acetone, ether, and chloroform on the hemolytic reaction in vitro. It is hoped to extend this work to cover many other substances such as aceto-acetic acid, beta oxybutyric acid, etc., which are found under pathologic conditions, and also perhaps to cover some of the aminoacids. The group under discussion are also being tried out with reference to their effect on antibody-production and on hemagglutinins and bacterial agglutinins.

EFFECT OF ETHER AND CHLOROFORM ANESTHESIA ON RABBIT COMPLEMENT IN VIVO

Three series of rabbits were selected and the C_H^+ and the complement titer determined for each rabbit. The animals in Series 1 were then anesthetized with ether for varying lengths of time and the C_H^+ and complement content of their sera again determined. The animals in Series 2 were saturated with sodium bicarbonate by intravenous, subcutaneous, and intraperitoneal injections of a 2% solution of sodium bicarbonate, and the C_H^+ and complement content of their sera determined. The animals were then put under ether anesthesia for varying lengths of time, and the C_H^+ and complement content again determined. Series 3 was given chloroform for varying lengths of time, and data obtained as before. The results were as follows:

The hydrogen-ion concentrations[12] were determined by the colorometric method,[13] the gas-chain method not being available. To 1 c.c. of boiled double-distilled water in a hemolytic test tube was added 1 drop of the indicator, the tubes were shaken, then 0.1 c.c. of the serum was added, and the tubes were again shaken, and read.

Since a hydrogen-ion concentration (C_H^+) of 10^{-7} is a truly neutral solution — that is the concentration of the hydroxyl ions (C_{OH}) is 10^{-7}— it will be noticed that rabbit blood seems to be slightly alkaline (10^{-8}) or neutral (10^{-7}) normally. Following ether anesthesia there is an increase in acidity. A drop in complement seemed to occur only when this increase in acidity passed the neutral point. In Series 2 the injection of sufficient sodium bicarbonate to change the C_H^+ from 10^{-7} to 10^{-10} did not cause any variation in the complement content. Neither

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[12] Washburn, Proc. Illinois Water Supply Assn., 1910, p. 93.

[13] Kelley, Jour. Lab. and Clin. Med., 1915, 1, p. 194. Wilson and Hutchinson, Jour. Biol. Chem., 1909, 4, 346.

TABLE 1

SERIES 1, ETHER ANESTHESIA

Rabbit	C_H^+	Complement Titer, Undiluted Serum (c.c.)	Time of Anesthesia (min.)	C_H^+	Complement Titer, Undiluted Serum (c.c.)
1	10^{-8}	0.05	10	10^{-6}	0.10
2	10^{-9}	0.03	15	10^{-7}	0.03
3	10^{-8}	0 03	20	10^{-6}	0.15
4	10^{-8}	0.08	45	10^{-5}	0.25

TABLE 2

SERIES 2, SODIUM BICARBONATE AND ETHER ANESTHESIA

Rabbit	C_H^+	Complement Titer (c.c.)	Material Injected (Sodium Bicarbonate)	C_H^+	Complement Titer (c.c.)	Time of Anesthesia (min.)	C_H^+	Complement Titer (c.c.)
1	10^{-7}	0.05	20 c.c. 2% solution	10^{-10}	0.05	30	10^{-7}	0.05
2	10^{-7}	0.03	20 c.c.	10^{-9}	0.03	45	10^{-65}	0.08
3	10^{-7}	0.03	1/12 gr. morphin	10^{-8}	0.03	15	10^{-8}	0.05
4	10^{-7}	0.05	40 c.c.	10^{-10}	0.05	30	10^{-7}	0.05
5	10^{-6}	0.30	30 c.c.	10^{-8}	0.05	15	10^{-7}	0.05

TABLE 3

SERIES 3, CHLOROFORM

Rabbit	C_H^+	Complement Titer (c.c.)	Time of Anesthesia (min.)	C_H^+	Complement Titer (c.c.)
1	10^{-8}	0.03	10	10^{-7}	0.03
2	10^{-8}	0.03	30	10^{-5}	0.40
3	10^{-8}	0.05	15		Killed
4	10^{-7}	0.05			Killed

did the complement titer drop when those animals which had been saturated with sodium bicarbonate were given ether anesthesia for varying lengths of time. The hydrogen-ion concentration did not return much past the neutral point of 10^{-7}. It is suggestive that Rabbit 5 had very little complement and a C_H^+ of 10^{-6}; after the animal had been saturated with sodium bicarbonate, the complement content returned to normal and the C_H^+ dropped to 10^{-8}. It has been stated that morphin prevents acidosis. Rabbit 3, Series 2, which received 1/12 gr. of morphin and then ether anesthesia, showed only a slight drop in complement content; the C_H^+ remained on the alkaline side of 10^{-7}, being 10^{-8} These experiments, while not conclusive, are suggestive.

EFFECT OF LACTIC ACID AND HYDROCHLORIC ACID ON COMPLEMENT IN VIVO

For this purpose 4 rabbits were selected, 2 for treatment with lactic acid and 2 for treatment with hydrochloric acid. The complement content and C_H^+ for each were determined and then 2 received each the equivalent of 0.5 c.c. of N/1 lactic acid intravenously, and 1 c.c. intraperitoneally, and the other 2 received each corresponding amounts and equivalents of N/1 hydrochloric acid. The C_H^+ and complement content were then determined and greater amounts of material injected. The results were as given in Table 4.

TABLE 4

DATA ON RABBITS INJECTED WITH LACTIC ACID

Rabbit	C_H^+	Complement Titer (c.c.)	Amount of Lactic Acid Injected (c.c.)	C_H^+	Complement Titer (c.c.)
1	10^{-7}	0.10	1.5	10^{-7}	0.08
	10^{-7}	0.05	6.0	10^{-7}	0.05
	10^{-7}	0.05	4.0	10^{-7}	0.05
2	10^{-7}	0.08	1.5	10^{-7}	0.05
	10^{-7}	0.05	6.0	10^{-7}	0.05
	10^{-7}	0.05	4.0	10^{-7}	0.05

The injection of lactic acid did not cause a drop in complement; it rather favored an increase in the complement content. The fact that the body is able to oxidize many of the organic acids, including lactic acid, over to the carbonates and that they then act as alkalies, would account for the similarity of action between lactic acid and sodium bicarbonate.

Twenty-four hours after these data were obtained, both animals received additional doses of lactic acid; death resulted. In administering the acid the desired amount of normal solution was pipetted out and diluted 15 or 20 times with isotonic salt solution, and this diluted solution injected.

The results of the action of hydrochloric acid are given in Table 5.

TABLE 5

DATA ON RABBITS INJECTED WITH HCl

Rabbit	C_H^+	Complement Titer (c.c.)	Material and Amount Injected	C_H^+	Complement Titer (c.c.)	Remarks
1	10^{-8}	0.03	5 c.c. N/20 HCl	10^{-65}	0.10	
	10^{-65}	0.10	15 c.c. N/20 HCl	10^{-6}	0.15	
	10^{-6}	0.15	20 c.c. 2% Na_2HCO_3			Died
2	10^{-8}	0.05	5 c.c. N/20 HCl	10^{-7}	0.10	
	10^{-7}	0.10	15 c.c. N/20 HCl	10^{-6}	0.15	
	10^{-6}	0.15	20 c.c. 2% Na_2HCO_3			Died

The results are in keeping with the fact that the body is unable to oxidize the mineral acids. The drop in complement did not occur immediately, but several hours after the administration of the acid. Normal variation in the rabbit complement might also account for a part, at least, of the variation. The complement might be affected also by any CO_2 liberated as the result of injection of dilute HCl and its action on the carbonates present in the body. Accordingly, fresh complement was taken and CO_2 bubbled through it for varying lengths of time up to 15 minutes. The hemolytic reaction was inhibited as long as CO_2 was present in an appreciable amount, but proceeded as soon as the CO_2 was driven off.

Since in a typical acidosis, such as one finds in diabetes, aceto-acetic acid, beta oxybutyric acid, acetone, and CO_2 are increased in the blood stream, it was next decided to try out acetone, aceto-acetic acid and beta oxybutyric acids not being available. Two rabbits were each given an injection of a 2% aqueous solution of acetone every hour for several hours, 20 c.c. being given at each injection. The C_H^+ and complement content were determined before and after each injection and on the half hour between. The C_H^+ remained from the beginning at 10^{-8} for Rabbit 1 and at 10^{-7} for Rabbit 2, and the complement titer did not vary from the original normals; that is, 0.03 c.c. contained 1 unit in Rabbit 1 and 0.05 c.c., 1 unit in Rabbit 2. Quantitative determinations of the amount of acetone in the blood stream or urine were not made. Again, these results might be explained on the basis of a not sufficient concentration of acetone in the blood stream, as well as that acetone by itself has no effect on complement in vivo. The former is a plausible explanation in view of the work of Manwaring, Hektoen, and others on the antilytic action of various salts when the concentrations were found to be important in vitro.

EFFECT OF DIFFERENT CONCENTRATIONS OF THE REAGENTS ON THE HEMOLYTIC REACTION

It was next decided to determine the effect of different concentrations of lactic acid, hydrochloric acid, uric acid, benzoic acid, tartaric acid, urea, hydrazin sulfate, acetic acid, acetone, ether, and chloroform on the hemolytic reaction.

In doing this it was thought necessary to determine the effect, if any, of each of these reagents on red blood cells, complement, and amboceptor, respectively. In working with lactic and hydrochloric acids, normal and decinormal solutions were carefully made up and standardized, and, when necessary, dilu-

TABLE 6

EFFECT OF THE VARIOUS REAGENTS ON HUMAN BLOOD CELLS

Reagent	Amount (c.c.) Used and Results			
	1	2	3	4
N/25 lactic acid	0.5 —	0.4 —	0.3 —	0.2 —
N/30 HCl	0.5 —	0.4 —	0.3 —	0.2 —
Saturated solution of uric acid	0.8 ++++	0.7 ++++	0.6 ++++	0.5 ++++
M/1 solution of urea	0.8 ++++	0.7 ++++	0.6 ++++	0.5 ++++
Saturated solution of benzoic acid	0.3 —	0.2 —	0.1 —	0.09 —
M/10 tartaric acid	0.2 —	0.1 —	0.09 —	0.08 —
1:10 dilution of saturated solution of hydrazin sulfate	0.4 —	0.3 —	0.2 —	0.1 —
M/10 acetic acid	0.5 —	0.4 —	0.3 —	0.2 —
Acetone, undiluted	0.5 —	0.4 —	0.3 —	0.2 —
Ether	0.5 —	0.4 —	0.3 —	0.2 —
Chloroform*	0.5 —	0.4 —	0.3 —	0.2 —

tions of these were carefully made. In the case of uric acid, benzoic acid, and hydrazin sulfate, on account of their slight solubilities, saturated solutions at 37 C. were used. It would perhaps have been better to have made up fractional parts of their molecular solutions based on their solubilities. In dealing with urea and tartaric acid, M/1 and M/10 solutions were carefully made in isotonic salt solution. The antihuman hemolytic system was used, with rabbit complement. Titrations of amboceptor and complement were made daily. One unit of amboceptor and 1 unit of complement were used in the tests for determining the effect of the reagents on amboceptor and complement respectively. Thirty-minute incubations at 37.5 C. in a water bath were used.

Various concentrations of each of the reagents were used in combination with red blood cells and salt solution to determine whether the substance was hemolytic, and if so, in what concentrations. Next, various concentrations were added to tubes containing salt solution and 1 unit of amboceptor, and the whole incubated 30 minutes at 37.5 C. in a water bath. Several volumes of salt solution were then added to bring the concentration below that which of itself would produce hemolysis, red blood cells were added, and the tubes shaken and again incubated to permit of the union of amboceptor and blood cells. The tubes were then removed and centrifugated, and the cells washed several times with salt solution. Enough salt solution plus 1 unit of comple-

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* Even Tubes 8, 9, and 10 have an excess of chloroform which would eventually hemolyze the blood cells. For accurate quantitative work on red blood cells and complement less

TABLE 6—*Continued*

EFFECT OF THE VARIOUS REAGENTS ON HUMAN BLOOD CELLS

Amount (c.c.) Used and Results							
5	6	7	8	9	10	11	12
0.1 +++	0.09 ++++	0.08 ++++	0.05 ++++	0.03 ++++	0.02 ++++	0.01 ++++	0.00 ++++
0.1 —	0.08 —	0.05 —	0.03 —	0.02 +++	0.01 ++++	0.00 ++++	
0.4 ++++	0.3 ++++	0.2 ++++	0.1 ++++	0.08 ++++	0.05 ++++	0.03 ++++	0.00 ++++
0.4 ++++	0.3 ++++	0.2 ++++	0.1 ++++	0.08 ++++	0.05 ++++	0.02 ++++	0.00 ++++
0.08 —	0.06 —	0.04 —	0.03 +	0.02 ++++	0.01 ++++	0.00 ++++	
0.07 —	0.06 +	0.05 ++	0.04 +++	0.03 ++++	0.02 ++++	0.01 ++++	0.00 ++++
0.08 —	0.05 —	0.04 —	0.03 +	0.02 ++++	0.01 ++++	0.00 ++++	
0.1 ±	0.08 +	0.06 ++	0.04 +++	0.03 ++++	0.02 ++++	0.01 ++++	0.00 ++++
0.1 ++++	0.08 ++++	0.06 ++++	0.04 ++++	0.02 ++++	0.01 ++++	0.00 ++++	
0.1 —	0.08 +	0.05 ++++	0.03 ++++	0.02 ++++	0.01 ++++	0.00 ++++	
0.1 —	0.08 —	0.05 —	0.03 ++	0.02 +++	0.01 ++++	0.00 ++++	

than these amounts of chloroform should be used. Blood cells from the author were not hemolyzed in the time mentioned; cells from another individual in the laboratory were.

ment was then added to bring the volume to 1 c.c., and the tubes were replaced in the water bath. In no case was there observed any effect exerted on amboceptor by any of the reagents used.

In testing the effect on complement 1 unit of complement was placed in each test tube along with salt solution, various concentrations of the reagent under investigation added, and the whole incubated 30 minutes. Red blood cells and amboceptor were then added and the tubes re-incubated. Controls were made in which the red blood cells and amboceptor had been permitted to unite before adding the reagents, and also controls were made for the hemolytic system. Data on the hemolytic activity had already been obtained. In the following tables varying degrees of hemolysis are indicated by the symbols used in the Wassermann reaction; a minus sign indicates complete hemolysis, and 4 plus signs indicate absence of hemolysis.

All the reagents included in Table 6, with the exception of uric acid and urea, were able to bring about laking of human red blood cells. Uric acid is a very weak acid, and this together with its very slight solubility may be important factors in eliminating it from the

TABLE 7

EFFECT OF THE VARIOUS REAGENTS ON COMPLEMENT

Reagent	Amount (c.c.) Used and Results			
	1	2	3	4
N/25 lactic acid	0.5 —	0.4 —	0.3 +++	0.25 ++++
N/30 HCl	0.4 —	0.3 —	0.2 —	0.15 ++
Saturated solution of uric acid	0.8 —	0.7 —	0.6 —	0.5 —
M/1 urea	0.5 ++++	0.4 ++++	0.3 ++++	0.2 +++
Saturated solution of benzoic acid	0.4 —	0.3 —	0.2 —	0.1 ++++
M/10 tartaric acid	0.4 —	0.3 —	0.2 —	0.1 —
1:10 dilution of saturated solution of hydrazin sulfate	0.4 —	0.3 —	0.2 —	0.15 +
M/10 glacial acetic acid	0.5 —	0.4 —	0.3 —	0.2 +
Acetone, undiluted	0.2 —	0.15 +	0.10 ++++	0.09 ++++
Ether	0.05 ++++	0.04 ++++	0.03 ++++	0.02 +++
Chloroform	0.03 ++++	0.02 ++++	0.01 ++++	0.00 —

group of hemolyzers. Urea, while comparatively soluble, is slightly basic in reaction, and apparently does not have an affinity for either the stroma or the hemoglobin of the cell. The group of hemolytic substances mentioned in this table contains mineral acids, fatty acids, aromatic acids, monohydroxy and dihydroxy acids, acetone, ether, chloroform, and hydrazin sulfate.

DISCUSSION

Little is known as to the exact mechanism of hemolysis. Moore[13] and others, in working with fatty acids and soaps, arrived at the general conclusion that hemolytic agents depend on the presence of a double bond uniting 2 carbon atoms. While this might hold true for the unsaturated fatty acids and soaps, the reagents under discussion in this paper do not fit in with his observations. Mathews,[14] Lilly,[15] and others, mention that ether and chloroform are hemolytic agents, and it

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[14] Phys. Chem., 1915, p. 924.

[15] Jour. Exper. Zool., 1914, 16, p. 591.

TABLE 7—*Continued*

EFFECT OF THE VARIOUS REAGENTS ON COMPLEMENT

Amount (c.c.) Used and Results							
5	6	7	8	9	10	11	12
0.2 ++++	0.15 +++	0.1 ±	0.08 —	0.06 —	0.04 —	0.02 —	0.00 —
0.10 ++++	0.09 ++++	0.08 ++++	0.07 +++	0.05 —	0.03 —	0.01 —	0.00 —
0.4 —	0.3 —	0.2 —	0.1 —	0.08 —	0.05 —	0.02 —	0.00 —
0.1 —	0.08 —	0.05 —	0.03 —	0.01 —	0.00 —		
0.09 ++++	0.08 ++++	0.07 ++++	0.06 —	0.05 —	0.04 —	0,02 —	0.00 —
0.08 +++	0.06 ++++	0.05 ++++	0.04 ++++	0.02 ++	0.01 —	0.00 —	
0.1 ++++	0.09 ++++	0.08 ++++	0.07 +++	0.05 —	0.03 —	0.01 —	0.00 —
0.1 ++++	0.08 ++++	0.06 ++++	0.04 ++++	0.02 +	0.01 —	0.00 —	
0.08 ++++	0.07 +++	0.06 +	0.05 —	0.03 —	0.02 —	0.01 —	0.00 —
0.01 +	0.00 —						

has long been known that mineral acids would act accordingly. So far as known, no report has been made on the remaining substances mentioned in this paper.

Mathews[16] has very clearly summed up the more recent views held as to the way hemolytic agents bring about their results. He states:

> "Hemoglobin may be held in the corpuscle by union with the stroma. It is true for all other cells, and probably it is true for the corpuscles, that they are not bags filled with fluid, but they are organized jellies. The corpuscles behave in many ways as if they also were such jellies. Hemoglobin does not escape as one would expect it would if it were in solution, when the corpuscle is punctured or cut across, but it stays in the divided corpuscle. Moreover, when hemoglobin is set free in the corpuscle by some of these methods, particularly in the very large cells of Necturus, a tailed amphibian, the hemoglobin may crystallize in the corpuscle itself, which shows that it must be prevented in some way from crystallizing in the normal cell. Moreover, the concentration of hemoglobin in the mammalian corpuscle is greater than the solubility of oxyhemoglobin in an equal bulk of water. For these and other reasons some observers are of the opinion that hemoglobin is held in some kind of a loose chemical or physical union, presumably the former, with the

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[16] Phys. Chem., 1915, p. 497.

stroma of the corpuscle and that the various hemolytic agents break this union. It is not at all impossible that the union is with certain reserve valencies of the hemoglobin and the stroma and such unions are very unstable and easily broken."

It is also quite probable that all the reagents do not act in exactly the same way. The color of the supernatant fluid resulting from the action of oxidizing agents, such as the acids, on the red blood cells is greenish probably because of the predominance of biliverdin, whereas the color following hemolysis by chloroform is more nearly a cherry red, similar to that following the action of immune sera.

It might be worthy of note in discussing the reagents used in this work that the lactic acid was of the racemic form, as is all lactic acid produced synthetically. This differs from the sarcolactic acid found in the body in that it is composed of both d-lactic and l-lactic acid, whereas the latter is d-lactic acid only. There might be some difference, but it is probable, in view of the general hemolytic properties of acids, that it would be a quantitative one only. The racemic form of lactic acid is found in the intestinal tract, and under pathologic conditions, may be present in the stomach. Sarcolactic acid is normally present in the tissues of the body and is especially increased during fatigue. The other hydroxyacid, tartaric, is dihydroxysuccinic acid, found in wine and some fruits, which are probably the source of this acid. Benzoic acid, an aromatic acid, is also taken into the body in fruits and berries. Bacterial decomposition may lead to the formation of phenol carbonic acid (benzoic acid) from the proteins by a series of oxidation reactions. Acetic acid, a fatty acid, is also produced in the intestinal tract as a result of bacterial fermentation. In a typical acidosis there are present aceto-acetic acid, acetone, and b-oxybutyric acid.

Hydrazin sulfate was studied because it was used by Dick[17] to produce necrosis of liver cells in dogs under observation for complement content. This probably breaks up into hydrazin and sulfuric acid. It is quite possible that hemolysis may result from the hydrazin-sulfate molecule, as well as from the action of the dissociation products.

None of these reagents is as strongly hemolytic as the unsaturated fatty acids, which will act in dilutions many times greater than any of the dilutions in which the other reagents mentioned are active.

One point in addition as to the mode of action of these hemolytic agents, is that, if the red blood cells are colloidal jellies, contact with free hydrogen ions will permit of a somewhat greater intake of water, and this, according to Brahmachari,[18] materially increases the amount of hemolysis.

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[17] Jour. Infect. Dis., 1913, 12, p. 111.

An inspection of the table showing the effect of the various reagents on complement shows that all except uric acid were capable of preventing hemolysis. Sachs and Altman[19] (1908), working with unsaturated fatty acids and soaps, showed that these were able to prevent hemolysis by hemolytic sera, and concluded that the action was that of an anticomplement. Moore, Wilson, and Hutchinson, in reviewing this work, speak of the action as a balancing action of hemolyzers; they do not believe that these reagents act as anticomplements. They say in part:

> "This result has been stated to be due to the neutralizing of complement, the sodium oleate acting as an anti-complement. We think, however, that there is clear evidence against this view. In the first place, as we shall see later, an *ordinary serum* which is not hemolytic to the corpuscles being used, it may, in fact, be their own serum, is strongly protective against the hemolytic action of sodium oleate. We have followed this question up in detail, as shown by the protocols of our experiments and have successively removed or destroyed (a) immune body, (b) complement, and (c) the lipoids from the active serum. In all cases we have found that no one of these substances is alone responsible for the neutralizing of the hemolytic activity of the sodium oleate. That is to say, in the active serum the immune body and the sodium oleate or linoleate combine and mutually destroy each other's laking power, so that the mixture in due proportion is quite inert upon the blood corpuscles."

The importance of this citation will be evident upon a close inspection of Tables 6 and 7. In the first place urea, which is not a hemolyzer, nevertheless prevents hemolysis by amboceptor and complement. This cannot be a balancing action of hemolyzers. Moreover, of the hemolytic agents which fix complement, the zone of fixation very closely approximates, and in many cases partially coincides with, the weakest dilutions producing hemolysis, and in many cases extends down to include a few which are unable to produce hemolysis. This is a phenomenon very similar to the one mentioned by Moore, Wilson, and Hutchinson. They do not seem to have taken into consideration the fact that the "ordinary serum" they mention as inhibiting the action of their hemolytic agent contained complement. They intimate that complement did take some part in the inhibiting action. After careful investigation of the substances mentioned in Tables 6 and 7, it was finally decided that, while other colloids present in fresh normal serum might take some part, the thermolabil colloid called complement played a large part. Whether in the nonhemolytic concentrations that fixed complement the effect was on the midpiece or endpiece of complement, was not investigated. It is a well-known fact that in the Wassermann reaction the midpiece alone is bound, the endpiece remaining free, in the supernatant fluid.

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[18] Jour. Biol. Chem., 1909, 4, p. 280.

[19] Berl. klin. Wchnschr., 1908, 10, p. 494.

I have shown also that CO_2 prevents complement from combining with amboceptor, but does not prevent amboceptor from combining with the red blood cell. This confirms the work of Sawtschenko,[20] who showed that in reality the midpiece unites with amboceptor, but that the endpiece is unable to unite with the midpiece. As soon as the CO_2 is removed, the reaction occurs. As Mathews says, this is probably one of the means the body has of preventing hemolysis in vivo by hemolytic sera.

Hektoen and Ruediger's work with the inorganic salts showed that these salts act as anticomplements in which the ions of Ca and Ba, etc., combine with the complement, giving Ca-complement, Ba-complement, etc.

The molecule of ether is not broken up in the animal body, as is that of chloroform. Graham[21] showed that in the blood stream chloroform ($CHCl_3$) is broken up into $COCl_2$ (Phosgene) and HCl. It would then appear that there are several possibilities for the fixing of complement by chloroform, as all these would possibly be present in the serum removed from an anesthetized animal.

The work of Lillie,[15] McClendon,[22] and others, is of interest because it suggests that the anesthetics may, in certain concentrations, play a double rôle in preventing hemolysis by amboceptor and complement. Lillie has observed that anesthetics prevent the outward diffusion of cell pigment produced by salts and thinks this a direct proof that anesthetics prevent increase in cell permeability. In order to determine positively whether the anesthetics were able to destroy or deflect complement in addition to this effect on the cell membrane, the following experiment was made:

One unit of complement (0.1 c.c.), 0.65 c.c. of physiologic salt solution, and 0.05 c.c. of ether were added to each of 3 hemolytic test tubes. These tubes were incubated 15 minutes at 37.5 C. and 0.1 c.c. of a 10% suspension of washed human red blood cells and 1 unit (0.1 c.c.) of antihuman hemolytic amboceptor added. The tubes were again incubated for 15 minutes in the water bath, centrifugated, and the supernatant fluids from all 3 tubes pipetted into a shallow dish, over which a current of air was passed to evaporate the ether. The centrifugated red blood cells in each of the three tubes were washed repeatedly with isotonic salt solution. After the last washing the supernatant fluids were removed and wasted; 0.9 c.c. of physiologic salt solution was then added to Tube 1, and 0.8 c.c. to Tube 2 together with 1 unit of complement; to Tube 3 was added 0.9 c.c. of the original supernatant fluid from which the ether had been evaporated. A 4th tube was then placed in the rack and to it were added 0.1 c.c. of a 10% suspension of washed human red blood cells and 0.9 c.c. of supernatant fluid similar to that added to Tube 3. The tubes were then incubated at 37.5 C. in a water bath for 30 minutes.

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[20] Ann. de l'Inst. de Pasteur, 1912, 26, p. 1032.

[21] Jour. Exper. Med., 1915, 22, p. 48.

[22] Am. Jour. Physiol., 1915, 38, p. 173.

The results were no hemolysis in Tubes 1 and 4, and complete hemolysis in Tubes 2 and 3. Hence it is evident that amboceptor had combined with the red blood cells in the presence of the anesthetic, and that all the complement had been deflected but not destroyed. In a future paper a report will be made as to the action of the various reagents on endpiece, midpiece, and the so-called third component of complement mentioned by Ehrlich,[23] Coca,[24] and others. These results are of interest in view of the inhibiting effect of ether on phagocytosis in vitro and the lowered resistance following anesthesia as shown by Graham.[25] He found that the opsonic power of an etherized serum was restored by evaporating the ether. My work would suggest as an explanation of his results that the ether prevented the complement from combining with the opsonic immune body. The negative results which he obtained for bacteriolysins are not comparable, owing to the necessity of using lower concentrations of ether in these experiments than in those on phagocytosis. They do suggest that in the animal body it is necessary to take into consideration other factors such as acidosis, etc., in addition to the anesthetic itself in order to account for all of the lowered resistance.

If data were available it would be interesting to tabulate for the various secretions, excretions, body fluids, and organ content the amount, if any, of each of these and other reagents present normally, as well as the amount under pathologic conditions, and compare these with the amount necessary to deflect or destroy complement. This might throw light on some acute and chronic infections, as well as on some focal infections. For example, normally the urine contains from 0.005 gm. to 0.02 gm. of urea per cubic centimeter; from Table 2 it may be shown that 0.012 gm. of urea is enough to fix 1 unit of complement. These data might be an explanation of the discrepancy of reports as to the presence or absence of complement in normal urine. It is conceivable that a cystitis might become chronic as a result of the presence of chemical substances interfering with phagocytosis. More recently[26] it has been shown that urea is increased in the blood during typhoid fever, especially in the severe cases. Mathews says that under certain pathologic conditions, such as interference with kidney function, the urea may increase to such an extent as to crystallize out on the skin on evaporation of perspiration. Urea may also be increased materially in the saliva, and particularly in the secretions of the duodenum and of the intestine.

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[23] Studies in Immunity, 1906.

[24] Ztschr. f. Immunitätsf., 1914, 21, p. 604.

[25] Jour. Infect. Dis., 1911, 8, p. 147.

Uric acid under normal conditions is present in about 0.00003 gm. per cubic centimeter of blood; under pathologic conditions this may be increased to 0.00015 gm. per cubic centimeter of blood. The amount present in the maximal amount of saturated solution used was only 0.000025 gm., which is one-sixth the amount which may be present under pathologic conditions. Hence, while complement is not affected by the amount used, it may well be that complement is deflected or destroyed by the same amount present under certain pathologic conditions.

From these data it is also clear how hyperacidity of the stomach may favor a chronic infection of the stomach. The same may be true for the intestinal tract in general.

It is also conceivable that an accumulation of by-products of metabolism in any organ or tissue may similarly lower the resistance of that organ or tissue so as to lead to a localization of infection there. On the other hand, it was noticed that many of the reagents studied seemed to hasten the hemolytic reaction when the concentrations used were much less than those fixing complement; that is, in many cases these tubes hemolyzed before the controls on the hemolytic system did. Hence it is conceivable that in certain weak concentrations many of these organic compounds may increase the efficiency of the immunity mechanism of the body, while in greater concentrations they may act in the reverse manner. It would be well worth while to work out the effect of as many of these reagents as possible on bacteriolysins, opsonins, etc., and also to determine whether it is possible to get a summation of effects, or whether anything acts as a protective substance, interfering in the body with the possible harmful effect of one or more of these compounds.

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[26] Jouve-Belmelle, Progrès Méd., 1916, 31, p. 149. Abstracted Jour. Am. Med. Assn., 1916, 67, p. 1189.

SUMMARY AND CONCLUSIONS

An apparent drop in complement often occurs as a result of anesthesia. This, however, is not constant.

The apparent drop probably is the result of the presence of the anesthetic, as well as of a slight increase in acidity.

Sodium bicarbonate and lactic acid injected into rabbits did not cause a drop in complement; if anything, they caused an increase. The lactic acid was probably oxidized over into carbonates by the body, thereafter acting as an alkali.

HCl injected into rabbits caused a marked drop in complement. This is as might be expected, since the body is unable to oxidize the mineral acids.

Acetone injected in relatively large amounts failed to cause a drop in complement.

Carbon dioxid, lactic acid, HCl, urea, benzoic acid, tartaric acid, acetic acid, acetone, ether, chloroform, and hydrazin sulfate deflect or destroy hemolytic complement in certain concentrations.

All these reagents, except urea and CO_2, bring about laking of human red blood cells in certain concentrations.

In general it may be said that the zone of concentrations fixing complement very nearly approximates, and in many cases coincides with, the weakest dilutions producing hemolysis, and may extend down to include a few concentrations which are unable to produce hemolysis.

The amount present in the blood is normally many times less than the amount required to affect complement. Under some pathologic conditions the concentration in the blood may be greatly increased, nearly approaching the amount which would affect complement.

On a rich protein diet the amount of urea normally in urine may exceed the amount necessary to fix complement.

This work suggests the possibility that the power of various organic compounds to deflect or fix complement may be a factor favoring infection or maintaining a chronic infection.

In addition to the selective action of many bacteria for certain tissue as the explanation of their localization therein (Rosenow), there may be also the fact of a lowered resistance of the organ or tissue due to increase of products of metabolism therein.

In very weak concentrations — that is, concentrations much too weak to fix complement — many of these reagents seem to accelerate the hemolytic reaction. This is analogous to the physiologic action of many pharmacologic products. Acting in this manner they might increase the efficiency of the immunity mechanism of the body.

At least in the case of the anesthetics there is a two-fold mechanism inhibiting hemolysis by amboceptor and complement — the action of the anesthetic on the permeability of the membrane, and the deflection of complement.

A saturated solution of uric acid in physiologic salt solution (37 C.) did not hemolyze red blood cells or fix complement. This concentration is however only one-sixth of that found under certain pathologic conditions.

None of these reagents in the concentrations studied prevented amboceptor from combining with red blood cells.

At least in the case of CO_2, ether, and chloroform, complement was not destroyed; but part or all of it was deflected.

This work suggests the possibility that some organic compounds might occasionally play some rôle in either inhibiting or intensifying the anaphylactic shock, since complement seems to be involved in this reaction.